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# 刚地弓形虫14-3-3蛋白真核表达载体的构建与表达

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# Construction and Expression of an Eukaryocyte Vector of 14-3-3 Protein in *Toxoplasma gondii*

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摘要 目的 构建并表达刚地弓形虫RH株14-3-3蛋白的真核表达载体。 方法 用生物信息学方法对弓形虫14-3-3蛋白的理化性质和结构进行预测。以弓形虫RH株总RNA为模板，逆转录PCR（RT-PCR）扩增目的基因片段，亚克隆至真核表达载体pcDNA3.0，构建重组质粒pcDNA3.0/14-3-3，经PCR、双酶切和测序鉴定正确后，用脂质体法转染人宫颈癌（HeLa）细胞，蛋白印迹（Western blotting）分析表达产物。 结果 根据14-3-3蛋白基因片段序列和氨基酸序列预测，该蛋白分子为酸性可溶性蛋白，主要以同源或异源二聚体存在，有5个氨基酸保守序列区。RT?鄄PCR的扩增产物约为800 bp，构建的真核表达质粒pcDNA3.0/14-3-3插入片段经测序，片段长度为801 bp，与GenBank中刚地弓形虫14-3-3蛋白基因序列（登录号为AB012775.1）同源性为99%。Western blotting分析结果显示，在转染pcDNA3.0/14-3-3的细胞中，有14?鄄3?鄄3蛋白表达，相对分子质量（Mr）约为30 000，且表达量显著高于转染空质粒和未转染的细胞。结论 构建了真核表达载体pcDNA3.0/14-3-3，并能在真核细胞内表达。

关键词： 刚地弓形虫 14-3-3蛋白 真核表达 生物信息学

Abstract： Objective To construct and express the eukaryotic expression vector of 14-3-3 protein of *Toxoplasma gondii* RH strain. Methods The structure and physicochemical property of 14-3-3 protein were predicted by bioinformatics analysis tools. The desired gene fragment was amplified from total RNA in *T. gondii* RH strain by RT-PCR, and sub-cloned into pcDNA3.0 to construct recombinant plasmid pcDNA3.0/14-3-3. After PCR confirming, double restriction enzyme digestion and DNA sequencing, the eukaryotic expression vector pcDNA3.0/14-3-3 was transfected into HeLa cells and the target protein was detected by Western blotting. Results The prediction of its gene sequence and amino acid sequence suggested that the 14-3-3 protein was acid soluble protein with five conserved regions, existing as homo- or hetero-dimers. The amplified gene fragment was about 800 bp, and the inserted fragment in pcDNA3.0/14-3-3 was 801 bp by sequencing, which had 99% homology to the 14-3-3 gene sequence of *T. gondii* in GenBank（Accession No. ABO12775.1）. Western blotting showed that there was more 14-3-3 protein expressed in the pcDNA3.0/14-3-3 transfected HeLa cells than untransfected and mock transfected cells. Its relative molecular mass （$M_r$） was about 30 000. Conclusion The eukaryotic expression vector pcDNA3.0/14-3-3 is constructed and expressed in eukaryotic cells.

Keywords： *Toxoplasma gondii* 14-3-3 protein Eukaryotic expression Bioinformatics

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